UNCLASSIFIED

DEFENCE R&D DÉFENSE

The Interaction of Chemical Agents and Therapeutic Compounds with Drugs used During Anaesthesia

John D. Conley
Kinchyle Enterprises Inc.

Scientific Authority:
P. Lundy
Defence Research Establishment Suffield

WARNING

"The use of this information is permitted subject to recognition of proprietary and patent rights."

DEFENCE RESEARCH ESTABLISHMENT SUFFIELD

Contract Report
DRES CR 2001-003

January 2001

National Defence Défense nationale

Canada

UNCLASSIFIED

THE INTERACTION OF CHEMICAL AGENTS AND THERAPEUTIC COMPOUNDS WITH DRUGS USED DURING ANAESTHESIA

W7702-7-R627/001/EDM AMENDMENT #003

Kinchyle Enterprises Inc.

48 Chinook Drive SouthWest
Medicine Hat, Alberta T1A 7M6
CANADA

Tel: (403) 527-8696
Fax: (403) 526-3833
E-mail kinchyle@memlane.com

THE INTERACTION OF CHEMICAL AGENTS AND THERAPEUTIC COMPOUNDS WITH DRUGS USED DURING ANAESHESIA
W7702-7-R627/001/EDM AMENDMENT #003

Index

Page 01	cover
Page02	index
Page 03-04	background, objectives, and scope of work
Page 04	scope of work (cont'd)
Page 05	summary
Page 06-10	animal model, anaesthetic technique, and experimental protocol
Page 11	milestones- *Determination of Toxicity*
Page 12-13	milestones-*Assessment of Anaesthetic requirements in OP-intoxicated Animals vs Control animals* *- HI 6*
Page 14-15	milestones- *Neuromuscular Blocking Agents (NMB)-Control Values*
Page 16-19	milestones- *Influence of Sarin (GB) on Neuromuscular Blocking Agents (NMB)*
Page 20-24	milestones- *Influence of Pyridostigmine Pretreatment on Neuromuscular Blocking Agents (NMB)*
Page 25-30	milestones- *Influence of Pyridostigmine Pretreatment Sarin Intoxication on Neuromuscular Blocking Agents (NMB)*
Page 31-33	milestones- *Determination of the Physiological Effects of Intramuscular (IM) Antidote in OP intoxicated Animals*
Page 34-37	milestones-*The Effect of Sulfur Mustard (HD) on Cardiovascular Respiratory Function* *- Effect of Ketamine Anaesthesia* *- Effect of HD Exposure during Halothane Anaesthesia* *- Effect of HD Exposure during Halothane Anaesthesia* *- Effect of Ketamine in HD-Intoxicated Swine* *- Effect of HD-Exposure during Ketamine Anaesthesia*
Page 38	milestones- *Collection of Epidermal Dermal Samples for Histopathological Assessment post-Reactive Skin Decontaminant Lotion (RSDL)*
Page 39-43	milestones- *The Effect of OP Intoxication on the Action of Intravenous Induction agents*
Page 44	milestones- *Collection of Blood for Evaluation of Possible Dyscrasias post-OP post-Therapeutic Agents*
Page 45	milestones- *Collection of Thoracic Contents for Analysis of Potential Free Radical Condensates*
Page 46-49	milestones- *Determination of IV Dose to Maintain Therapeutic Levels post-Autoinjector (AI)*
Page 50	acknowledgments

THE INTERACTION OF CHEMICAL AGENTS AND THERAPEUTIC COMPOUNDS WITH DRUGS USED DURING ANAESTHESIA
W7702-7-R627/001/EDM AMENDMENT #003

Background:

Historically, plans for the treatment of battlefield casualties have tended to polarize around either conventional casualties or chemical casualties Recent world events have highlighted the possibility of combined casualties (military &/or civilian).

Canadian scientists at Defence Research Establishment Suffield (DRES) have recognized the conjectural nature of treatment plans for combined casualties

Objectives:

This contract examines one aspect of the management of mixed casualties .specifically the management of surgical patients who require both a general anaesthetic (GA) and who:

01)-have received prophylactic treatment (pyridostigmine) for anticipated exposure to chemical weapons (CW).
Or
02)-have been exposed to CW.
Or
03)-have received current antidote post-CW exposure
Or
04)-have received combinations of the above

It is the aim of this study

01)-to identify potential adverse drug interactions with anesthetic drugs currently in the Canadian Forces (CF) formulary
02)-to suggest suitable anaesthetic management regimens
03)-to highlight areas requiring further critical examination

Scope of Work:

To meet the objectives of this contract, an animal model used by other friendly nations has been adapted and refined

The following milestones have been completed

01)-determination of the toxicity of a representative organophosphate (OP) in the animal model
02)-measurement/collation/analysis of appropriate physiological parameters in both control and CW exposed animals
03)-assessment of anaesthetic requirements in OP-intoxicated vs. control animals
04)-determination of effects of pyridostigmine pretreatment on neuromuscular blocking agents (NMB) in both non-OP-intoxicated animals and OP-intoxicated animals
05)-determination of the physiological effects of IM antidote (AI) in OP-intoxicated animals
06)-determination of the effect of sulfur mustard (HD) on cardiovascular/respiratory function
07)-collection of epidermal/dermal samples for histopathological assessment (time-saline control/post-reactive skin decontaminant lotion-post-RSDL).
08)-assessment of effects of OP intoxication on intravenous induction agents
09)-collection of blood for evaluation of possible dyscrasias post-OP/post-therapeutic agents

10)-collection of thoracic contents for analysis of potential free radical generating condensates.

THE INTERACTION OF CHEMICAL AGENTS AND THERAPEUTIC COMPOUNDS WITH DRUGS USED DURING ANAESHESIA
W7702-7-R627/001/EDM AMENDMENT #003

11)-conversion of physiological data to Excel and Prism formats
12)-determination of IV dose of HI-6 to maintain therapeutic levels post-AI
13)-other
-Appendix A

THE INTERACTION OF CHEMICAL AGENTS AND THERAPEUTIC COMPOUNDS WITH DRUGS USED DURING ANAESHESIA
W7702-7-R627/001/EDM AMENDMENT #003

Summary:

01)-IV sarin (GB) in a dose of 9 μg/kg consistently results in signs of OP intoxication and depression of cholinesterase activity (Ch_{ase}) to approximately 15% of normal in a domestic swine model (DSM).
02)-the following physiological parameters were measured in all animals
- A)-heart rate (HR)
- B)-mean direct arterial blood pressure (MAP)
- C)-pulse oximetry (S_pO2)
- D)-capnography ($P_{ET}CO2$)
- E)-respiratory frequency (f)
- G)-core temperature (°C)
- H)-neuromuscular junction activity (NMJ)

03)-the following physiological parameters were measured in some animals.
- A)-electrocardiogram (EKG)
- B)-electroencephalogram (EEG)
- C)-additional respiratory parameters
  - i) tidal volume (V_t)
  - ii) minute ventilation (V_E)
  - iii) airway resistance (R_{aw})
  - iv) dynamic compliance (Cl_{dyn})
  - v) work of breathing (WoB)

04)-Al/HI-6 IV appears to change anaesthetic requirements
05)-HD interacts adversely with ketamine and succinylcholine (SDC)
06)-GB consistently depresses total, true and pseudo-cholinesterase activity
07)-pyridostigmine produces a transient elevation in platelet counts and decreases bleeding time
08)-the following adverse anaesthetic drug interactions are noted in GB-intoxicated animals
- A)-SDC
  - i) adequate relaxation may not be produced by usual iv doses
  - ii) if adequate relaxation occurs, duration of paralysis may be prolonged
- B)-vecuronium (VEC)
  - i) adequate relaxation occurs at higher doses
  - ii) duration of paralysis may be lengthened at higher doses
- C)-sodium thiopental (STP)
  - i) depressant effects appear exaggerated
- D)-ketamine
  - i) depressant effects appear exaggerated
- E)-propofol
  - i) appears to act normally
- F)-pyridostigmine
  - i) prolongs the duration of action of SDC
  - ii) antagonizes the action of VEC

THE INTERACTION OF CHEMICAL AGENTS AND THERAPEUTIC COMPOUNDS WITH DRUGS USED DURING ANAESHESIA
W7702-7-R627/001/EDM AMENDMENT #003

Animal Model:

The animal chosen for this series of experiments is the domestic swine (*Sus scrofa domestica*). Castrated male animals (Yorkshire-Landrace cross) of 9-10 weeks of age and weighing approximately 20 kg are utilized

Justification:

01)-acceptability-The domestic swine model (DSM) is acceptable to the end-user (Canadian Forces medical/paramedical personnel) because of extensive utilization in biomedical research. Anesthetists, in particular, are aware of the importance of the DSM in Malignant Hyperthermia Syndrome (MHS) and in pharmacological research into NMB drugs In the latter case, DSM is the best non-primate model for human response. Additionally, the DSM has been promoted as a model for the study of anesthetics. The DSM has been promoted as model for dermatological research because of similarities to humans

02)-availability-The animals are obtained from a dependable local supplier familiar with the experimental requirements Animal costs have remained fixed and compare favorably to costs of other animals commonly utilized in biomedical research. Quality and health have remained consistently high.

03)-adaptability-The size of animals allows easy technical manipulation and facilitates utilization of readily available human surgical instrumentation, anaesthetic equipment and monitoring devices The cardiovascular and pulmonary systems of pigs are morphologically and functionally similar to humans and pigs are large enough to allow for frequent blood sampling and reliable monitoring of central haemodynamics and lung mechanics
There are no special handling considerations beyond indoor housing and feeding using commercial feed The weight range chosen is approximately 1/3rd that of the stereotypical 70 kg man, allowing a reasonable approximation of human doses i e 1 autoinjector (AI) to a compromised pig equals the maximum dose deliverable to a compromised 70 kg human

04)-precedence-The DSM has been utilized in the past for research with sarin (GB), soman (GD) and HI 6/atropine allowing comparison with already collected/published data

In conducting this research the authors adhered to the "Guide to the Care and Use of Experimental Animals" and "The Ethics of Animal Experimentation" published by the Canadian Council on Animal Care

The Animal Care Committee (ACC) at DRES approved the experimental protocol prior to the commencement of experimentation.

Drugs:

Justification:

Drugs utilized are those found in the formulary of the Canadian Forces (CF) A notable exception is the volatile anaesthetic agent halothane, which was used because of ease of inhalation induction and because of relative cost compared to isoflurane

Nitrous oxide (N_2O) was utilized at the time of induction to facilitate the induction (2nd Gas Effect) and to reduce costs.

Isoflurane in the carrier gas oxygen (O_2) was used as the control anaesthetic the sole exception to this was the utilization of halothane in the HD experiments

THE INTERACTION OF CHEMICAL AGENTS AND THERAPEUTIC COMPOUNDS WITH DRUGS USED DURING ANAESHESIA
W7702-7-R627/001/EDM AMENDMENT #003

The AI preparation is that developed for the CF. Because of difficulties obtaining issue binary autoinjectors, equivalent dosages of atropine (2 mg) and HI-6 (500 mg) were given intramuscularly (im).

Monitors:

Justification.

Basic monitors utilized are those recommended by the Canadian Anesthesiologists' Society (CAS) for monitoring during general anesthesia (intubated) Additional monitors utilized during some experiments include electroencephalography (EEG) and pulmonary dynamics monitoring (Bicore Pulmonary Dynamics Monitor).

Aside from the exceptions noted below, most of the equipment utilized was either already present at DRES or was purchased "used".

The **Bicore Pulmonary Dynamics Monitor** was purchased new under a different contract, but was utilized with the kind permission of the scientific authority (SA), Dr. S.Duncan

THE INTERACTION OF CHEMICAL AGENTS AND THERAPEUTIC COMPOUNDS WITH DRUGS USED DURING ANAESHESIA
W7702-7-R627/001/EDM AMENDMENT #003

Anesthesia:

Animals were fed until the evening prior to surgery and allowed tap water *ad libitum* until the time of the experiment. The animals were not premedicated. After weighing, all animals underwent an inhalation induction in the transport container. Halothane (3%) in a carrier gas mixture of oxygen (O_2) and nitrous oxide (N_2O) in a ratio of 1:1 and at a flow rate of 8 lpm was administered utilizing a Boyle anesthesia machine with a circle patient anesthesia circuit Once induced the animal was placed in the dorsal recumbent (supine) position on a heated operating table and the halothane concentration reduced to 2% in 100% O_2 at a flow rate of 1 lpm Animals were intubated with a 6 0 mm internal diameter (I.D) cuffed oral endotracheal tube (Ruschelit, Willy Rusch AG, 71394 Kernen, Germany) and the cuff was inflated with room air to achieve a seal. Central intravenous access was established by means of a cut-down into the right external jugular vein A 20 gauge IV catheter was inserted into the right carotid artery for blood pressure measurement and blood sampling. A tracheotomy was performed, the endotracheal tube removed and a 6 0 mm ID cuffed tracheotomy tube (Ruschelit, Willy Rusch AG, 71394 Kernen, Germany) inserted All catheters were fixed to the skin and all incisions closed
Three 0 5-ml samples of arterial blood for control cholinesterase activity (Ch_{esc}) were drawn at 5-minute intervals Halothane was discontinued and isoflurane 2% in 100% O_2 at a flow rate of 1 lpm substituted After a 20 minute equilibration period a further three 0 5 ml samples of arterial blood for control cholinesterase activity (Ch_{esc}) were drawn at 5 minute intervals. Baseline physiological parameters were recorded simultaneously.
Concomitantly with the anesthetic, all animals received IV normal saline (sodium chloride 0.9%, Abbott Laboratories Ltd) at a rate of 8 ml/kg/hr utilizing a volumetric infusion pump (Travenol FloGard 8000, Travenol laboratories). Urinary output was measured via a percutaneously placed needle cystostomy

THE INTERACTION OF CHEMICAL AGENTS AND THERAPEUTIC COMPOUNDS WITH DRUGS USED DURING ANAESHESIA
W7702-7-R627/001/EDM AMENDMENT #003

Monitors.

A pulse oximeter and capnographic sensor were connected to a multifunction monitor (Nellcor N1000) which was in turn connected via analog outputs to a Coulbourn Instruments high speed videograph I/O port (L19-02). To measure blood pressure the catheter in the right carotid artery was connected to a transducer (Cobe CDXpress 3cc) which, in turn, was connected to a Coulbourn Instruments type A transducer coupler (S72-75) Body temperature was recorded by a rectal thermistor probe (Coulbourn Instruments temperature coupler S71-30) and was maintained at approximately 38.5 °C by means of a Sage-London Industries Inc Therm-o-matic heated operating table Cardiac rate and rhythm were recorded via a Coulbourn Instruments isolated bioamplifier (S75-05). Neuromuscular junction activity was stimulated using supramaximal stimuli produced by a NeuroTechnology Microstim Plus anesthesia nerve stimulator and was recorded using a Coulbourn Instruments T45-10 accelerometer connected to a Type A transducer coupler (S72-75) A simple electroencephalographic trace was obtained by surgical implantation of screw electrodes into cortical bone of the skull supraorbitally 1-cm lateral to the midline and by percutaneous placement of a single stainless steel ground needle in the neck musculature Recordings were made utilizing a Coulbourn Instruments high gain bioamplifier with bypass filter (S75-01). Data was collected via a Coulbourn Instruments LabLinc S computer interface to An ALR 386 IBM compatible PC. Data was displayed and stored using WinGraph for Windows software.
Airway pressure and flow were measured at the inflow end of the tracheotomy tube utilizing a VarFlex flow transducer (Bear Medical Systems, Inc, Riverside, CA) Esophageal pressure was measured utilizing an 8FR SmartCath catheter (Bear Medical Systems, Inc, Riverside, CA) Proper positioning of the intraesophageal balloon was confirmed utilizing an occlusion test. These transducers were connected to a Bicore CP-100 adult pulmonary monitor (Bear Medical Systems, Inc, Riverside, CA) Data was collected via a serial output to an ALR FlexCache SX 386Z IBM compatible PC

THE INTERACTION OF CHEMICAL AGENTS AND THERAPEUTIC COMPOUNDS WITH DRUGS USED DURING ANAESHESIA
W7702-7-R627/001/EDM AMENDMENT #003

Experimental Protocol:

After the monitors were established the animals were allowed a stabilization period of at least 30 min, during which time steady-state anesthesia was established Baseline values were obtained prior to drug administration and measurements were continued for the duration of anesthesia and displayed on a standard CRT monitor. These measurements included direct arterial pressure, heart rate, EKG, temperature, EEG, respiratory frequency (f), SpO2 and $P_{ET}CO2$. The following respiratory parameters were derived using the Bicore CP-100 adult pulmonary monitor: tidal volume (V_t), minute ventilation (V_E), airway resistance (R_{aw}), dynamic lung compliance (Cl_{dyn}) and work of breathing (WoB_p). Total infused fluids and total urine output were also recorded.

Blood was collected throughout the experimental period for ACh_{ase} determination by the method of Johnson and Russell

All animals were sacrificed at the end of the experiment and while still receiving a general anaesthetic (GA) with either an overdose of sodium pentobarbital (3400 mg) or intra-arterial air embolus

THE INTERACTION OF CHEMICAL AGENTS AND THERAPEUTIC COMPOUNDS WITH DRUGS USED DURING ANAESHESIA
W7702-7-R627/001/EDM AMENDMENT #003

Milestones:

Determination of Toxicity

(Animal Reference-97005-011, 98015-028,030, 032-046)

It was determined at the outset that the selected dose of agent should produce significant morbidity in the DSM without significant mortality. This level of compromise would then simulate the "worst case" scenario post-triage in which treatment would be "urgent" rather than "expectant".

Sarin (GB) was selected by the scientific authority (SA) at DRES as the representative organophosphate (OP) chemical weapon (CW) to utilize over the course of the contract Sulfur mustard (HD) was the vesicant chosen. Both agents were synthesized at DRES. Neat agent was handled/diluted by DRES personnel

After review of the available literature it was determined that the selected dose of GB should

01)-be administered intravenously (IV)
02)-result in a depression of Ch_{ase} activity to the level found in human victims of GB intoxication whose injuries were classified as "severe" i.e. 10-15%
03)-result in significant clinical signs

GB was administered by slow IV "push" over a 10-min interval in a range of 4-9 :g/kg

It was demonstrated that 9 µg/kg of GB produced consistent depression of Ch_{ase} activity to the target value of 10-15% and reproduced the following clinical signs

01)-bradypnea often progressing to apnea
02)-mastication
03)-miosis
04)-neuromuscular phenomena ranging from fasciculations to generalized convulsions
05)-bradycardia
06)-hypotension
07)-ororrhea, rhinorrhea, bronchorrhea

THE INTERACTION OF CHEMICAL AGENTS AND THERAPEUTIC COMPOUNDS WITH DRUGS USED DURING ANAESHESIA
W7702-7-R627/001/EDM AMENDMENT #003

Assessment of Anesthetic requirements in OP-intoxicated Animals vs. Control animals:

MAC in swine is greater than in humans MAC values for isoflurane using the "standard" tail-clamp technique described by Eger et al in 1965 is between 1 45+/-0.17% and 1 65+/-0.36%.

A standard anaesthetic was administered except where specifically noted. This anesthetic consisted of 2% isoflurane in a carrier gas of 100% oxygen (F_IO2=1.0) administered at a flow rate of one liter per minute (1 lpm). The assumption was made these animals received comparable levels of GA approximating 1 MAC at equilibrium.

No formalized attempt was made to assess changes in Minimum Alveolar Concentration (MAC) associated with experiments for the following reasons:

01)-infrared spectrometry is required
02)-classical assessment of MAC requires movement in response to a noxious stimulus Such movement is difficult to assess in the presence of neuromuscular phenomena associated with OP intoxication and the muscle relaxation associated with anesthetic neuromuscular blocking drugs (NMB)

The following opinions are offered.

01)-the neuromuscular phenomena associated with GB intoxication and the depth of GA maintained for the experimental period would preclude all but the crudest of surgical procedures
02)-the clinical solution to 01) would be to
i)-"deepen" the anaesthetic
or
ii)-paralyze the patient using NMB drugs

Both of these solutions have the potential for adverse drug interactions and outcome. Further research in this area is suggested.

HI 6

There are neuromuscular phenomena associated with the administration of intramuscular (im) atropine (2 0 mg)/HI 6 (500 mg) and/or the intravenous administration of HI 6 in doses between 10 mg/hr-60 mg/hr Animals were noted to fasciculate and have sometimes violent non-purposeful movement These phenomena have been reported previously

The following opinions are offered.

01)-the neuromuscular phenomena associated with atropine/HI 6 &/or HI 6 and the depth of anesthesia maintained for the experimental period would preclude all but the crudest of surgical procedures
02)-the clinical solution to 01) would be to.
i)-"deepen" the anaesthetic (?efficacy)
or
ii)-paralyze the patient using NMB drugs

Both of these solutions have the potential for adverse drug interactions and outcome. Further research in this area is suggested.

THE INTERACTION OF CHEMICAL AGENTS AND THERAPEUTIC COMPOUNDS WITH DRUGS USED DURING ANAESHESIA
W7702-7-R627/001/EDM AMENDMENT #003

The question of whether or not these phenomena would occur in an *unanesthetized animal* has not been addressed. Further research in this area is suggested.

The question of whether or not these phenomena would occur in an *animal under Regional Block* has not been addressed. Further research in this area is suggested.

THE INTERACTION OF CHEMICAL AGENTS AND THERAPEUTIC COMPOUNDS WITH DRUGS USED DURING ANAESHESIA
W7702-7-R627/001/EDM AMENDMENT #003

Neuromuscular Blocking Agents (NMB)-Background.

NMBs are used in anesthesia to facilitate endotracheal intubation and for surgical relaxation Their major site of action is the nicotinic receptor at the muscle membrane of the neuromuscular junction (NMJ).

Depolarizing NMBs like succinylcholine (SCh) are acetylcholine (ACh) agonists. The type of blockade produced by SCh is the same type produced by OPs Normally, the binding of SCh to receptor sites at the NMJ causes a depolarization that persists longer than that caused by ACh because Acetylcholinesterase (AChE) does not hydrolyze SCh. Paralysis occurs because a second action potential cannot occur as long as the membrane is still depolarized. SCh is rapidly metabolized by pseudocholinesterase (plasma cholinesterase) accounting for its short duration of action.

A nondepolarizing NMB like vecuronium (VEC) is competitive antagonists for ACh and prevents the binding of ACh to its receptors at the NMJ The resultant blockade may be "reversed" by carbamates like neostigmine or pyridostigmine. Carbamates act by reversibly binding to AChE, thus resulting in more ACh at the NMJ.

Neuromuscular Blocking Agents (NMB)-Control Values

Two important considerations are.
i)-the time of onset of neuromuscular blockade
ii)-the duration of the neuromuscular blockade

Time of onset of neuromuscular blockade in this experiment was determined to be the time when apnea occurred. This has clinical significance as apnea is often used to determine the timing of endotracheal intubation especially during a rapid-sequence or "crash" induction. This latter technique is designed to protect the airway from a potentially fatal aspiration of gastric contents and would be used in the emergency management of casualties

Requirements for surgical relaxation vary. Choice of NMB may be predicated upon the duration of neuromuscular blockade required. Reversal of blockade is a prerequisite to the "recovery" of the patient from anesthesia Incomplete reversal of neuromuscular blockade jeopardizes patient safety and may be associated with morbidity and/or mortality In this experiment, recovery from neuromuscular blockade was deemed to have occurred when ET_{CO2} within 5 mm Hg of values obtained prior to the administration of the NMB.

Intravenous (iv) intubating doses for swine of SDC and VEC are 1 mg/kg and 0.15 mg/kg respectively In keeping with common clinical practice, a standardized dose of 20 mg for SCh and 3 mg for VEC was utilized Both drugs were administered by "iv push" over 60 sec.

Table – Time to Apnea (sec) Following SCh (20 mg iv)-Control

DOSE #	# OF TESTS	AVERAGE+/- 1SD	MEDIAN	RANGE
1st	26	47.8 +/- 13.3	43	27-85
2nd	26	49.4 +/- 17.2	44	30-110
3rd	25	48 5 +/- 12.7	47	35-75

THE INTERACTION OF CHEMICAL AGENTS AND THERAPEUTIC COMPOUNDS WITH DRUGS USED DURING ANAESHESIA
W7702-7-R627/001/EDM AMENDMENT #003

Table – Time to Recovery (sec) Following SCh (20 mg iv)-Control

DOSE #	# OF TESTS	AVERAGE+/- 1SD	MEDIAN	RANGE
1st	23	713 +/- 324	649	360-1801
2nd	26	722 +/- 191	683	437-1272
3rd	24	781 +/- 327	683	319-1760

Table – Time to Apnea (sec) Following VEC (3 mg iv)-Control

DOSE #	# OF TESTS	AVERAGE+/- 1SD	MEDIAN	RANGE
1st	15	77.5 +/- 32 8	79	38-158
2nd	8	68 1 +/- 19.9	67	37-94
3rd	2	72.5	72 5	56-89

Table – Time to Recovery (sec) Following VEC (3 mg iv)-Control

DOSE #	# OF TESTS	AVERAGE+/- 1SD	MEDIAN	RANGE
1st	14	959 +/- 202	902	622-1395
2nd	5	2265 +/- 2877	1122	610-7574
3rd	2	1225	1225	1200-1250

THE INTERACTION OF CHEMICAL AGENTS AND THERAPEUTIC COMPOUNDS WITH DRUGS USED DURING ANAESHESIA
W7702-7-R627/001/EDM AMENDMENT #003

Influence of Sarin (GB) on Neuromuscular Blocking Agents (NMB):

OPs irreversibly bind to AChE As a result, ACh is not hydrolyzed and accumulates at the NMJ producing persistent depolarization and hence paralysis. OPs also irreversibly bind to pseudocholinesterase

Since SCh is an ACh agonist, OP intoxication might lessen the amount of SCh required to produce a block More significantly, prolongation of the neuromuscular block might occur because of either inhibition of metabolism of SCh by pseudocholinesterase or relative overdose SCh resulting in a "phase II" block

VEC is a competitive antagonist of ACh. Since OPs may increase the concentration of ACh at the NMJ more VEC may required to produce relaxation Duration of relaxation may be lessened. Carbamates normally used to reverse neuromuscular blockade may not be efficacious.

Table – Time to Apnea (sec) Following SCh (20 mg iv)-post-GB (5μg/kg)

DOSE #	# OF TESTS	AVERAGE+/- 1SD	MEDIAN	RANGE
1st	2	120 sec/NO		
2nd	3	NO/70sec/NO		
3rd	3	NO/NO/NO		

Versus

Table – Time to Apnea (sec) Following SCh (20 mg iv)-Control

DOSE #	# OF TESTS	AVERAGE+/- 1SD	MEDIAN	RANGE
1st	26	47.8 +/- 13.3	43	27-85
2nd	26	49 4 +/- 17 2	44	30-110
3rd	25	48 5 +/- 12 7	47	35-75

SIGNIFICANCE-At this dose of GB (5 μg/kg), the usual intubating dose of SCh (1 mg/kg) did not produce apneic conditions. Endotracheal intubation *could be difficult to impossible.* This finding is *contrary to expectations* as OP intoxication would be expected to provide more agonist in the form of ACh at the NMJ and, thus, less SCh should be needed to produce neuromuscular blockade. GB and SCh appear to be *antagonistic.*

Table – Time to Recovery (sec) Following SCh (20 mg iv)-post-GB (5μg/kg)

DOSE #	# OF TESTS	AVERAGE+/- 1SD	MEDIAN	RANGE
1st	3	532 +/- 101	516	439-640
2nd	3	633 +/- 129	590	530-778
3rd	3	605 +/-52	600	556-660

THE INTERACTION OF CHEMICAL AGENTS AND THERAPEUTIC COMPOUNDS WITH DRUGS USED DURING ANAESHESIA
W7702-7-R627/001/EDM AMENDMENT #003

Versus

Table – Time to Recovery (sec) Following SCh (20 mg iv)-Control

DOSE #	# OF TESTS	AVERAGE+/- 1SD	MEDIAN	RANGE
1st	23	713 +/- 324	649	360-1801
2nd	26	722 +/- 191	683	437-1272
3rd	24	781 +/- 327	683	319-1760

SIGNIFICANCE-At this dose of GB (5 μg/kg), recovery from the usual intubating dose of SCh (1 mg/kg) was more rapid than controls. This finding is contrary to expectations as OP intoxication would be expected to prolong the action of SCh by inhibition of pseudocholinesterase or by production of a "phase II" block. OPs and SCh appear to be antagonistic.

Table – Time to Apnea (sec) Following SCh (20 mg iv)-post-GB (9μg/kg)

DOSE #	# OF TESTS	AVERAGE+/- 1SD	MEDIAN	RANGE
1st	5	78 6 +/- 46 5	62 0	44-160
2nd	6	69.8 +/- 23 0	62 5	50-113
3rd	6	57.8 +/- 19.2	51 5	41-95

Versus

Table – Time to Apnea (sec) Following SCh (20 mg iv)-Control

DOSE #	# OF TESTS	AVERAGE+/- 1SD	MEDIAN	RANGE
1st	26	47.8 +/- 13.3	43	27-85
2nd	26	49.4 +/- 17 2	44	30-110
3rd	25	48.5 +/- 12 7	47	35-75

SIGNIFICANCE-At this dose of GB (9 μg/kg) the time to apneic conditions following the usual intubating dose of SCh (1 mg/kg) is prolonged. Endotracheal intubation would be delayed placing the patient at risk to aspiration for a longer period. This finding is contrary to expectations as OP intoxication would be expected to provide more agonist in the form of ACh at the NMJ and, thus, less SCh should be needed to produce neuromuscular blockade. GB and SCh appear to be antagonistic.

Table – Time to recovery (sec) Following SCh (20 mg iv)-post-GB (9μg/kg)

DOSE #	# OF TESTS	AVERAGE+/- 1SD	MEDIAN	RANGE
1st	7	2346 +/- 2335	1713	548-7216
2nd	6	1124 +/- 386	925	765-1650
3rd	6	1503 +/- 941	1104	758-3210

THE INTERACTION OF CHEMICAL AGENTS AND THERAPEUTIC COMPOUNDS WITH DRUGS USED DURING ANAESHESIA
W7702-7-R627/001/EDM AMENDMENT #003

Versus

Table – Time to Recovery (sec) Following SCh (20 mg iv)-Control

DOSE #	# OF TESTS	AVERAGE+/- 1SD	MEDIAN	RANGE
1st	23	713 +/- 324	649	360-1801
2nd	26	722 +/- 191	683	437-1272
3rd	24	781 +/- 327	683	319-1760

SIGNIFICANCE-At this dose of GB (9 μg/kg), recovery from the usual intubating dose of SCh (1 mg/kg) is prolonged relative to controls. This finding is expected. OP intoxication would be expected to prolong the action of SCh by inhibition of pseudocholinesterase or by production of a "phase II" block. OPs and SCh appear to be agonistic.

Table – Time to Apnea (sec) Following VEC (3 mg iv)-post-GB (9 μg/kg)

DOSE #	# OF TESTS	AVERAGE+/- 1SD	MEDIAN	RANGE
1st	5	70/135/NO/NO/NO		
2nd	4	- /101/NO/NO/160		
3rd	2	- / - / - / NO/138		

Versus

Table – Time to Apnea (sec) Following VEC (3 mg iv)-Control

DOSE #	# OF TESTS	AVERAGE+/- 1SD	MEDIAN	RANGE
1st	15	77 5 +/- 32.8	79	38-158
2nd	8	68 1 +/- 19.9	67	37-94
3rd	2	72.5	72.5	56-89

SIGNIFICANCE-At this dose of GB (9 μg/kg) the time to apneic conditions following the usual intubating dose of VEC (0.15 mg/kg) is prolonged. Endotracheal intubation would be delayed placing the patient at risk to aspiration for a longer period. This finding as expected. OP intoxication would result in more competitive antagonist in the form of ACh at the NMJ. More VEC should be needed to produce neuromuscular blockade. It is suggested that the dose for intubation be increased by 1.5-2x. This suggestion requires confirmation.

Table – Time to Recovery (sec) Following VEC (3 mg iv)- post-GB (9 μg/kg)

DOSE #	# OF TESTS	AVERAGE+/- 1SD	MEDIAN	RANGE
1st	5	843 +/- 422	568	513-1320
2nd	4	1069 +/- 920	696	446-2437
3rd	2	645	645	522-769

THE INTERACTION OF CHEMICAL AGENTS AND THERAPEUTIC COMPOUNDS WITH DRUGS USED DURING ANAESHESIA
W7702-7-R627/001/EDM AMENDMENT #003

Versus

Table – Time to Recovery (sec) Following VEC (3 mg IV)-Control

DOSE #	# OF TESTS	AVERAGE+/- 1SD	MEDIAN	RANGE
1st	14	959 +/- 202	902	622-1395
2nd	5	2265 +/- 2877	1122	610-7574
3rd	2	1225	1225	1200-1250

SIGNIFICANCE-At this dose of GB (9 μg/kg), recovery from the usual intubating dose of VEC (0.15 mg/kg) is shortened relative to controls. This finding is *expected*. OP intoxication would be expected to increase the amount of competitive antagonist (ACh) to the neuromuscular blockade produced by VEC. OPs and VEC appear to be *antagonistic*.

THE INTERACTION OF CHEMICAL AGENTS AND THERAPEUTIC COMPOUNDS WITH DRUGS USED DURING ANAESHESIA
W7702-7-R627/001/EDM AMENDMENT #003

Influence of Pyridostigmine Pretreatment on Neuromuscular Blocking Agents (NMB)

Pyridostigmine (PYR) is a dimethylcarbamate used as a pretreatment against OP intoxication When there is threat of nerve agent exposure CF personnel may receive PYR 30 mg po q8h for up to 7 days.

Both PYR and OPs are anticholinesterases. The major difference is that PYR binds reversibly to the enzyme acetylcholinesterase (AChE) whereas OPs bind irreversibly. The prescribed prophylactic dose of PYR results in
approximately 30% reversible inhibition of AChE , leaving 70% of the enzyme unprotected and at risk to irreversible inhibition by OPs Reported side-effects are rare at this dosage

(Animal Reference-98024-030, 032-037, 050-052,060-062, 066, 067, 069)

SUCCINYLCHOLINE

Pyridostigmine 40 µg/kg iv

Animals 98-024, 026-029

Table Time to Apnea following 20 mg SCh-Pyridostigmine 40 µg/kg iv:

DOSE #	# OF TESTS	AVERAGE+/- 1SD	MEDIAN	RANGE
1st	5	40.6 +/- 5 9	39	35-50
2nd	5	41 6 +/- 5.6	43	35-49
3rd	4	42.3 +/- 7.0	43.5	33-49

NOTE. 1st dose of SCh given 15 min post-pyridostigmine

Versus

Table – Time to Apnea (sec) Following SCh (20 mg iv)-Control

DOSE #	# OF TESTS	AVERAGE+/- 1SD	MEDIAN	RANGE
1st	26	47 8 +/- 13.3	43	27-85
2nd	26	49 4 +/- 17.2	44	30-110
3rd	25	48 5 +/- 12.7	47	35-75

THE INTERACTION OF CHEMICAL AGENTS AND THERAPEUTIC COMPOUNDS WITH DRUGS USED DURING ANAESHESIA
W7702-7-R627/001/EDM AMENDMENT #003

SIGNIFICANCE-At this dose of Pyridostigmine(40 µg/kg), time to apnea following the usual intubating dose of SCh (1.0 mg/kg) is shortened relative to controls. This finding is *expected*. Because PYR pretreatment would be expected to increase the amount of agonist (ACh) at the NMJ, less SCh should be required to produce the neuromuscular blockade. PYR and SCh appear to be *agonistic*.

Table Time to Recovery following 20 mg SCh-Pyridostigmine 40 µg/kg iv:

DOSE #	# OF TESTS	AVERAGE+/- 1SD	MEDIAN	RANGE
1st	6	975 +/- 665	815	472-2235
2nd	6	853 +/- 1020	853	426-2406
3rd	5	976 +/- 296	890	650-1359

NOTE 1st dose of SCh given 15 min post-pyridostigmine

Versus

Table – Time to Recovery (sec) Following SCh (20 mg iv)-Control

DOSE #	# OF TESTS	AVERAGE+/- 1SD	MEDIAN	RANGE
1st	23	713 +/- 324	649	360-1801
2nd	26	722 +/- 191	683	437-1272
3rd	24	781 +/- 327	683	319-1760

SIGNIFICANCE-At this dose of PYR (40 µg/kg), recovery from the usual intubating dose of SCh (1.0 mg/kg) is prolonged relative to controls. This finding is *expected*. PYR would be expected to inhibit the metabolism of SCh. PYR and SCh appear to be *agonistic*.

Pyridostigmine 80 µg/kg iv

Because pyridostigmine 40 µg/kg iv did not produce consistent depression of AChE by 30%, the dose of PYR was increased to PYR 80 µg/kg iv

Animals 98-030, 032-037

THE INTERACTION OF CHEMICAL AGENTS AND THERAPEUTIC COMPOUNDS WITH DRUGS USED DURING ANAESHESIA
W7702-7-R627/001/EDM AMENDMENT #003

Table Time to Apnea following 20 mg SCh-**Pyridostigmine 80 μg/kg iv:**

DOSE #	# OF TESTS	AVERAGE+/- 1SD	MEDIAN	RANGE
1st	5	66.5 +/- 20.1	71.5	35-89
2nd	6	57.3 +/- 12.8	55	45-80
3rd	6	55 9 +/- 7.8	55	46-67

NOTE. 1st dose of SCh given 15 min post-pyridostigmine
One animal did not go apneic and is not included in the results

Versus

Table – Time to Apnea (sec) Following SCh (20 mg iv)-**Control**

DOSE #	# OF TESTS	AVERAGE+/- 1SD	MEDIAN	RANGE
1st	26	47.8 +/- 13.3	43	27-85
2nd	26	49.4 +/- 17.2	44	30-110
3rd	25	48.5 +/- 12 7	47	35-75

SIGNIFICANCE-At this dose of Pyridostigmine (80 μg/kg), time to apnea following the usual intubating dose of SCh (1.0 mg/kg) is prolonged relative to controls. This finding is *unexpected*. Because PYR pretreatment would be expected to increase the amount of agonist (ACh) at the NMJ, less SCh should be required to produce the neuromuscular blockade.

Table Time to Recovery following 20 mg SCh-**Pyridostigmine 80 μg/kg iv:**

DOSE #	# OF TESTS	AVERAGE+/- 1SD	MEDIAN	RANGE
1st	7	645 +/- 318	546	419-1324
2nd	6	863 +/- 347	679	599-1433
3rd	7	803 +/- 291	722	504-1266

NOTE. 1st dose of SCh given 15 min post-pyridostigmine

Versus

Table – Time to Recovery (sec) Following SCh (20 mg iv)-**Control**

DOSE #	# OF TESTS	AVERAGE+/- 1SD	MEDIAN	RANGE
1st	23	713 +/- 324	649	360-1801
2nd	26	722 +/- 191	683	437-1272
3rd	24	781 +/- 327	683	319-1760

THE INTERACTION OF CHEMICAL AGENTS AND THERAPEUTIC COMPOUNDS WITH DRUGS USED DURING ANAESHESIA
W7702-7-R627/001/EDM AMENDMENT #003

SIGNIFICANCE-At this dose of PYR (80 µg/kg), recovery from the usual intubating dose of SCh (1.0 mg/kg) is prolonged relative to controls only after the 2nd dose. This finding is *unexpected*. PYR would be expected to inhibit the metabolism of SCh and prolong the recovery from neuromuscular blockade.

VECURONIUM

Vecuronium controls:

Animals 98-048, 049, 056, 053-056, 057-059

Pyridostigmine 80 :g/kg iv

Animals 98-050-052, 060-062

Table – Time to Apnea (sec) Following VEC (3 mg iv)-post-Pyridostigmine 80 µg/kg iv

DOSE #	# OF TESTS	AVERAGE+/- 1SD	MEDIAN	RANGE
1st	5	62 2 +/- 15 7	61	40-84
2nd	2	67 5		45-90
3rd	1	61		

Note: 1st dose of VEC given 15 min post-PYR
1 animal following 1st , 2nd and 3rd dose did not become apneic and are not included in the results

Versus

Table – Time to Apnea (sec) Following VEC (3 mg iv)-Control

DOSE #	# OF TESTS	AVERAGE+/- 1SD	MEDIAN	RANGE
1st	15	77.5 +/- 32 8	79	38-158
2nd	8	68.1 +/- 19.9	67	37-94
3rd	2	72.5	72 5	56-89

SIGNIFICANCE-At this dose of PYR (80 µg/kg) the time to apneic conditions following the usual intubating dose of VEC (0.15 mg/kg) appear to be shortened if one does not take into account the animals which never became apneic. Endotracheal intubation would be delayed in the latter case placing the patient at risk to aspiration for a longer period. This latter finding as *expected*. PYR pretreatment should result in more competitive antagonist in the form of ACh at the NMJ. More VEC should be needed to produce neuromuscular blockade. It is suggested that the dose for intubation be increased by 1.5-2x. This suggestion requires confirmation.

THE INTERACTION OF CHEMICAL AGENTS AND THERAPEUTIC COMPOUNDS WITH DRUGS USED DURING ANAESHESIA
W7702-7-R627/001/EDM AMENDMENT #003

Table – Time to Recovery (sec) Following VEC (3 mg iv)- post-PYR (80 μg/kg)

DOSE #	# OF TESTS	AVERAGE+/- 1SD	MEDIAN	RANGE
1st	6	927 +/- 332	800	635-1455
2nd	3	1994 +/- 2170	788	695-4500
3rd	2	984		813-1155

1st dose of VEC given 15 mm post-PYR

Versus

Table – Time to Recovery (sec) Following VEC (3 mg iv)-Control

DOSE #	# OF TESTS	AVERAGE+/- 1SD	MEDIAN	RANGE
1st	14	959 +/- 202	902	622-1395
2nd	5	2265 +/- 2877	1122	610-7574
3rd	2	1225	1225	1200-1250

SIGNIFICANCE-At this dose of PYR (80 μg/kg), recovery from the usual intubating dose of VEC (0.15 mg/kg) is shortened relative to controls. This finding is *expected*. PYR pretreatment would be expected to increase the amount of competitive antagonist (ACh) to the neuromuscular blockade produced by VEC. PYR and VEC appear to be *antagonistic*.

THE INTERACTION OF CHEMICAL AGENTS AND THERAPEUTIC COMPOUNDS WITH DRUGS USED DURING ANAESHESIA
W7702-7-R627/001/EDM AMENDMENT #003

Influence of Sarin (GB) on Neuromuscular Blocking Agents (NMB):

OPs irreversibly bind to AChE. As a result, ACh is not hydrolyzed and accumulates at the NMJ producing persistent depolarization and hence paralysis. OPs also irreversibly bind to pseudocholinesterase.

Since SCh is an ACh agonist, OP intoxication might lessen the amount of SCh required to produce a block More significantly, prolongation of the neuromuscular block might occur because of either inhibition of metabolism of SCh by pseudocholinesterase or relative overdose SCh resulting in a "phase II" block.

VEC is a competitive antagonist of ACh. Since OPs may increase the concentration of ACh at the NMJ more VEC may required to produce relaxation Duration of relaxation may be lessened Carbamates normally used to reverse neuromuscular blockade may not be efficacious.

Influence of Pyridostigmine Pretreatment on Neuromuscular Blocking Agents (NMB)·

Pyridostigmine (PYR) is a dimethylcarbamate used as a pretreatment against OP intoxication When there is threat of nerve agent exposure CF personnel may receive PYR 30 mg p.o. q8h for up to 7 days

Both PYR and OPs are anticholinesterases The major difference is that PYR binds reversibly to the enzyme acetylcholinesterase (AChE) whereas OPs bind irreversibly The prescribed prophylactic dose of PYR results in
approximately 30% reversible inhibition of AChE , leaving 70% of the enzyme unprotected and at risk to irreversible inhibition by OPs Side effects are rare at this dosage.

Influence of Pyridostigmine Pretreatment Sarin Intoxication on Neuromuscular Blocking Agents (NMB)

Both GB and PYR would be expected to increase the amount of agonist (ACh) at the NMJ

Theoretically, the block produced by SCh should be facilitated whereas that produced by VEC should be antagonized

Both GB and PYR should inhibit the hydrolysis Of SCh and thus prolong the blockade produced The presence of increased ACh should antagonize the blockade produced by VEC

THE INTERACTION OF CHEMICAL AGENTS AND THERAPEUTIC COMPOUNDS WITH DRUGS USED DURING ANAESHESIA
W7702-7-R627/001/EDM AMENDMENT #003

Table – Time to Apnea (sec) Following SCh (20 mg iv)-post-PYR (40 μg/kg)/GB (5μg/kg)

DOSE #	# OF TESTS	AVERAGE+/- 1SD	MEDIAN	RANGE
1st	2	63.5 +/- 2 1		62-65
2nd	6	90 0 +/- 5o 1	76	46-186
3rd	6	64.5 +/- 22 7	62	38-105

Versus

Table – Time to Apnea (sec) Following SCh (20 mg iv)-Control

DOSE #	# OF TESTS	AVERAGE+/- 1SD	MEDIAN	RANGE
1st	26	47 8 +/- 13.3	43	27-85
2nd	26	49 4 +/- 17 2	44	30-110
3rd	25	48.5 +/- 12.7	47	35-75

SIGNIFICANCE-At this dose of PYR (40 μg/kg)/GB (5μg/kg) the time to apneic conditions following the usual intubating dose of SCh (1 mg/kg) is prolonged. Endotracheal intubation would be delayed placing the patient at risk to aspiration for a longer period. This finding is *contrary* to expectations as PYR/OP intoxication would be expected to provide more agonist in the form of ACh at the NMJ and, thus, less SCh should be needed to produce neuromuscular blockade. PYR/GB and SCh appear to be *antagonistic*.

Table – Time to Recovery (sec) Following SCh (20 mg iv)-post-PYR (40 μg/kg)/GB (5μg/kg)

DOSE #	# OF TESTS	AVERAGE+/- 1SD	MEDIAN	RANGE
1st	5	2829 +/- 1434	2228	1449-5100
2nd	6	1843 +/- 1276	1207	879-3900
3rd	5	2501 +/- 1831	1397	1045-4500

Versus

Table – Time to Recovery (sec) Following SCh (20 mg iv)-Control

DOSE #	# OF TESTS	AVERAGE+/- 1SD	MEDIAN	RANGE
1st	23	713 +/- 324	649	360-1801
2nd	26	722 +/- 191	683	437-1272
3rd	24	781 +/- 327	683	319-1760

SIGNIFICANCE-At this dose of PYR (40 μg/kg)/GB (5 μg/kg), recovery from the usual intubating dose of SCh (1 mg/kg) was longer than controls. This finding is *expected* as PYR/GB intoxication would be expected to prolong the action of SCh by inhibition of pseudocholinesterase or by production of a "phase II" block. PYR/OPs and SCh appear to be *agonistic*.

THE INTERACTION OF CHEMICAL AGENTS AND THERAPEUTIC COMPOUNDS WITH DRUGS USED DURING ANAESHESIA
W7702-7-R627/001/EDM AMENDMENT #003

Table – Time to Apnea (sec) Following SCh (20 mg iv)-post-PYR (80 µg/kg)/GB (5µg/kg)

DOSE #	# OF TESTS	AVERAGE+/- 1SD	MEDIAN	RANGE
1st	1	44		
2nd	1	59		
3rd	2	53		38-68

Note: 1 animal did not go apneic following the 2nd dose of SCh and is not included in results

Versus

Table – Time to Apnea (sec) Following SCh (20 mg iv)-Control

DOSE #	# OF TESTS	AVERAGE+/- 1SD	MEDIAN	RANGE
1st	26	47 8 +/- 13 3	43	27-85
2nd	26	49 4 +/- 17 2	44	30-110
3rd	25	48.5 +/- 12 7	47	35-75

SIGNIFICANCE-At this dose of PYR (80 µg/kg)/GB (5µg/kg) the time to apneic conditions following the usual intubating dose of SCh (1 mg/kg) is prolonged One animal did not go apneic. Endotracheal intubation would be delayed placing the patient at risk to aspiration for a longer period. This finding is *contrary* to expectations as PYR/OP intoxication would be expected to provide more agonist in the form of ACh at the NMJ and, thus, less SCh should be needed to produce neuromuscular blockade. PYR/GB and SCh appear to be *antagonistic*.

Table – Time to Recovery (sec) Following SCh (20 mg iv)-post-PYR (80 µg/kg)/GB (5µg/kg)

DOSE #	# OF TESTS	AVERAGE+/- 1SD	MEDIAN	RANGE
1st	2	712		705-718
2nd	2	1040		481-1599
3rd	2	1379		822-2186

Versus

Table – Time to Recovery (sec) Following SCh (20 mg iv)-Control

DOSE #	# OF TESTS	AVERAGE+/- 1SD	MEDIAN	RANGE
1st	23	713 +/- 324	649	360-1801
2nd	26	722 +/- 191	683	437-1272
3rd	24	781 +/- 327	683	319-1760

THE INTERACTION OF CHEMICAL AGENTS AND THERAPEUTIC COMPOUNDS WITH DRUGS USED DURING ANAESHESIA
W7702-7-R627/001/EDM AMENDMENT #003

SIGNIFICANCE-At this dose of PYR (80 µg/kg)/GB (5 µg/kg), recovery from the usual intubating dose of SCh (1 mg/kg) took longer than controls. This finding is *expected* as PYR/GB intoxication would be expected to prolong the action of SCh by inhibition of pseudocholinesterase or by production of a "phase II" block. PYR/OPs and SCh appear to be *agonistic*.

Table – Time to Apnea (sec) Following SCh (20 mg iv)-post-PYR (80 µg/kg)/GB (9µg/kg)

DOSE #	# OF TESTS	AVERAGE+/- 1SD	MEDIAN	RANGE
1st	1	100		100
2nd	4	70 8 +/-19 0	67	53-96
3rd	1	62		62

Versus

Table – Time to Apnea (sec) Following SCh (20 mg iv)-Control

DOSE #	# OF TESTS	AVERAGE+/- 1SD	MEDIAN	RANGE
1st	26	47.8 +/- 13.3	43	27-85
2nd	26	49 4 +/- 17 2	44	30-110
3rd	25	48 5 +/- 12 7	47	35-75

SIGNIFICANCE-At this dose of PYR (80 µg/kg)/GB (9µg/kg) the time to apneic conditions following the usual intubating dose of SCh (1 mg/kg) is prolonged. Endotracheal intubation would be delayed placing the patient at risk to aspiration for a longer period. This finding is *contrary* to expectations as PYR/OP intoxication would be expected to provide more agonist in the form of ACh at the NMJ and, thus, less SCh should be needed to produce neuromuscular blockade. PYR/GB and SCh appear to be *antagonistic*.

Table – Time to Recovery (sec) Following SCh (20 mg iv)-post-PYR (80 µg/kg)/GB (9µg/kg)

DOSE #	# OF TESTS	AVERAGE+/- 1SD	MEDIAN	RANGE
1st	4	5275 +/- 3757	5480	720-10800
2nd	3	2450 +/- 1880	2450	360-3900
3rd	1	2165		2165

THE INTERACTION OF CHEMICAL AGENTS AND THERAPEUTIC COMPOUNDS WITH DRUGS USED DURING ANAESHESIA
W7702-7-R627/001/EDM AMENDMENT #003

Versus

Table – Time to Recovery (sec) Following SCh (20 mg iv)-Control

DOSE #	# OF TESTS	AVERAGE+/- 1SD	MEDIAN	RANGE
1st	23	713 +/- 324	649	360-1801
2nd	26	722 +/- 191	683	437-1272
3rd	24	781 +/- 327	683	319-1760

SIGNIFICANCE-At this dose of PYR (80 μg/kg)/GB (9 μg/kg), recovery from the usual intubating dose of SCh (1 mg/kg) took much longer than controls. This finding is *expected* as PYR/GB intoxication would be expected to prolong the action of SCh by inhibition of pseudocholinesterase or by production of a "phaseII" block. PYR/OPs and SCh appear to be *agonistic*.

Table – Time to Apnea (sec) Following VEC (3 mg iv)-post-Pyridostigmine (80 :g/kg)/GB (9 μg/kg) iv

DOSE #	# OF TESTS	AVERAGE+/- 1SD	MEDIAN	RANGE
1st	2	65		50-80
2nd	2	45		40-50
3rd	2	42.5		40-45

Versus

Table – Time to Apnea (sec) Following VEC (3 mg iv)-Control

DOSE #	# OF TESTS	AVERAGE+/- 1SD	MEDIAN	RANGE
1st	15	77.5 +/- 32 8	79	38-158
2nd	8	68.1 +/- 19 9	67	37-94
3rd	2	72 5	72.5	56-89

SIGNIFICANCE-At this dose of PYR (80 μg/kg)/GB (9 μg/kg) the time to apneic conditions following the usual intubating dose of VEC (0.15 mg/kg) appear to be shortened. This latter finding as *unexpected*. PYR pretreatment should result in more competitive antagonist in the form of ACh at the NMJ. More VEC should be needed to produce neuromuscular blockade.

THE INTERACTION OF CHEMICAL AGENTS AND THERAPEUTIC COMPOUNDS WITH DRUGS USED DURING ANAESHESIA
W7702-7-R627/001/EDM AMENDMENT #003

Table – Time to Recovery (sec) Following VEC (3 mg iv)- post-PYR (80 µg/kg) GB (9 µg/kg)

DOSE #	# OF TESTS	AVERAGE+/- 1SD	MEDIAN	RANGE
1st	3	1295 +/- 485	1252	833-1800
2nd	2	1056		813-1299
3rd	1	1800		1800

1st dose of VEC given 15 min post-PYR

Versus

Table – Time to Recovery (sec) Following VEC (3 mg iv)-Control

DOSE #	# OF TESTS	AVERAGE+/- 1SD	MEDIAN	RANGE
1st	14	959 +/- 202	902	622-1395
2nd	5	2265 +/- 2877	1122	610-7574
3rd	2	1225	1225	1200-1250

SIGNIFICANCE-At this dose of PYR (80 µg/kg)/GB (9 µg/kg), recovery from the usual intubating dose of VEC (0.15 mg/kg) is variable relative to controls. This finding is *unexpected*. PYR pretreatment would be expected to increase the amount of competitive antagonist (ACh) to the neuromuscular blockade produced by VEC, thus shortening the duration of the blockade.

THE INTERACTION OF CHEMICAL AGENTS AND THERAPEUTIC COMPOUNDS WITH DRUGS USED DURING ANAESHESIA
W7702-7-R627/001/EDM AMENDMENT #003

Determination of the Physiological Effects of Intramuscular (IM) Antidote in OP-intoxicated Animals

(Animal Reference-97003, 006, 98010, 011, 015, 027, 028, 030-033, 039, 043, 073, 074, 086, 103, 99137-145, 147-151, 158-179)

During the course of our investigations we have noted significant disparities between our findings (unpublished) and work published by Nyberg et al..

In brief, they found that one autoinjector administered IM to anaesthetized domestic swine counteracted the signs of OP poisoning (soman-2xLD_{50}), obviated the need for ventilatory assistance and reactivated acetylcholmesterase (AChE).

In contrast, we have observed that one autoinjector (equivalent) administered IM to anaesthetized domestic swine did not counteract the signs of OP poisoning with GD, did not obviate the need for ventilatory assistance and did not reactivate AChE

There are both *similarities* and *differences* in the methodology.

Similarities in methodology:
01-castrated male domestic swine
02-weight approx 22 kg
03-similar instrumentation

Differences in methodology.

01-premedication	-azaperone IM (S)	vs unpremedicated (C)
02-anaesthetic agent	-ketamine (S)	vs. isoflurane (C)
03-GD dosage	-9 :g/kg IV (S)	vs 2 5 :g/kg IV (C)
04-GD infusion time	-over 20 mm (S)	vs over 5 min. (C)
05-antidote	-binary autoinjector (S)	vs intramuscular (C)
06-antidote timing	-5 min into GD infusion (S)	vs. with signs (C)

In the Swedish study animals received approximately 50 µg of GD prior to administration of the antidote, whereas in our study animals received slightly more than 50 µg of GD prior to administration of the antidote Animals in the Swedish study demonstrated signs of OP intoxication prior to the admmistration of the antidote.

Conclusion:
Pigs in the Swedish and Canadian studies received equivalent amounts of GD prior to the administration of antidote.

THE INTERACTION OF CHEMICAL AGENTS AND THERAPEUTIC COMPOUNDS WITH DRUGS USED DURING ANAESHESIA
W7702-7-R627/001/EDM AMENDMENT #003

Animals in the Swedish study received the antidote via a binary autoinjector supplied by the manufacturer Canadian animals received the identical drugs via an IM injection There are clear advantages to the autoinjector but studies in Sweden have demonstrated that a satisfactory level of HI-6 (4 $\mu g\ mL^{-1}$) is attained within 1 min following either conventional IM or autoinjector administration.

We have used autoinjectors (old) and IM administration of antidote in GB intoxicated animals with comparable rates of reactivation of AChE.

Conclusions:
The influence of route of administration on the observed differences in both morbidity and enzyme reactivation should be minimal.

The swine in the Swedish study were premedicated and received IV ketamine whereas the animals in the Canadian study had an inhalation induction with halothane and were subsequently maintained on isoflurane

Ketamine, *in vitro*, reversibly inhibits AChE and theoretically could prevent the action of an irreversible inhibitor like GD on AChE. Our data suggests a decrease in ChE activity with isoflurane general anaesthesia (compared to halothane controls)

The influence of ketamine as a NMDA channel blocker in this circumstance remains to be elucidated

The influence of azaperone premedication (if any) is unknown.

Conclusion:
The efficacy of the antidote with respect to both clinical course and AChE reactivation appears to be influenced by the anaesthetic agent.

THE INTERACTION OF CHEMICAL AGENTS AND THERAPEUTIC COMPOUNDS WITH DRUGS USED DURING ANAESHESIA
W7702-7-R627/001/EDM AMENDMENT #003

RECOMMENDATIONS:

Inappropriate anaesthetic agent selection for the care of patients who have been poisoned by OP agents may increase morbidity and mortality The effectiveness of the antidote issued to the Canadian Forces appears suspect under some conditions which might occur

It is suggested that further investigation be conducted to determine.

01-the influence of butyrophenone premedication on clinical course and enzyme reactivation.
Azaperone is not used in human anaesthesia. Droperidol should be considered.
02-the influence of IV ketamine on clinical course and enzyme reactivation

Suggested Animal Utilization:

01-compare the influence of IM azaperone premedication (3.3 mg/kg) vs no premedication in animals induced with halothane and converted to IV ketamine (0.5 mg/kg/min).
a) if azaperone has a significant effect, the experiment should be repeated using isoflurane
b) if azaperone has a significant effect with isoflurane, the experiment should be repeated with droperidol.

Premed (no/yes) → halothane x 60 min (ChE q5min) → maintenance anaesthetic x 90 min (ChE q5min) → GD (2.5 :g/kg/5 min)

→ Antidote (signs) → (ChE q30 sec x 5min, q5min until 30 min q 15 min until 5 hr post-GD)

EXPERIMENT #	CONDITIONAL UPON RESULTS	PREMED	MAINTENANCE	ANIMAL #
1	-	none	IV ketamine	3
2	-	azaperone	IV ketamine	3
3	1,2	azaperone	isoflurane	3
4	3	droperidol	isoflurane	3

Physiological parameters would be measured, collated and analyzed.

THE INTERACTION OF CHEMICAL AGENTS AND THERAPEUTIC COMPOUNDS WITH DRUGS USED DURING ANAESHESIA
W7702-7-R627/001/EDM AMENDMENT #003

The Effect of Sulfur Mustard (HD) on Cardiovascular Respiratory Function

Accepted for publication in ***Military Medicine***

(Animal Reference-98012-014, 077-085, 095-100, 99146)

This laboratory has had a continuing interest in sulphur mustard (bis (2-chloroethyl) sulphide, NATO Standard Agreement code: HD), a chemical warfare agent that has been well documented as being toxic to a variety of tissue and organ systems. Although, historically, its primary military use has been as a vesicant (blister causing) agent, it is also capable of exerting systemic effects on the immune, cardiovascular and central nervous systems at high concentrations

During studies designed to investigate the vesicant actions of this agent (unpublished data), it was noted that in ketamine-anaesthetized rats, topical challenges of HD (2 x 1 µL, ~ 200 g animals) caused signs of intoxication that closely mimicked those of organophosphate poisoned animals including: mastication, muscle fasciculation, straub tail and respiratory distress These animals often died, apparently of respiratory failure, even though similarly treated, but unanaesthetized animals never presented any signs of stress or discomfort

In an effort to further investigate the adverse interaction between HD and ketamine, the domestic swine model was utilised

Effect of Ketamine Anaesthesia:

After halothane steady-state anaesthesia for 30 min, an iv infusion of ketamine (0.5 mg/kg/min) was started and halothane exposure terminated No significant changes in respiratory frequency, arterial blood pressure, Sa_{O2}, ET_{CO2} or temperature were noted over the following two hours. Acetylcholinesterase activity was marginally elevated, while heart rate declined slightly so that 2 h after the initiation of ketamine anaesthesia, it was ~ 71 % of starting values. Urine output was 3.0 +/- 1 7 ml/kg/min.

Effect of HD Exposure during Halothane Anaesthesia.

Pigs were injected with HD (1mg/kg) after halothane steady state was achieved and changes in physiological parameters were followed over the ensuing 5 h. Heart rate, respiratory frequency, temperature and Sa_{O2} did not change significantly Blood pressure gradually fell over the next 1.5 h (~ 82 % of starting values) and then stabilised, while ET_{CO2} gradually increased over the five hour duration of the experiment (~ 160 %) Urine output was generally extremely depressed during these treatments. One animal died under this paradigm and the data derived during this experiment was not included. However, in this animal, urine output was 0.2 ml/kg/min. In the animals used for this group the urine output was 0 7, 0 5 and 4.0 ml/kg/min (mean +/- SD = 1 7 +/- 2.0 ml/kg/min). Acetylcholinesterase activity was inhibited (~ 50 %). Five hours post-HD, the animals were treated with 20 mg of SCh In every case a prolonged apnea of at least 30min was induced from which recovery was judged clinically to be improbable When compared to historical controls in which halothane anaesthetised animals were treated with SCh, the time to apnea and the time required for ET_{CO2} to decline to zero were marginally, but not significantly increased in HD-poisoned animals. In contrast, the time required to regain spontaneous breathing and to recovery by these animals was dramatically increased, so that clinical judgement suggested that they would not recover without active ventilatory support

THE INTERACTION OF CHEMICAL AGENTS AND THERAPEUTIC COMPOUNDS WITH DRUGS USED DURING ANAESHESIA
W7702-7-R627/001/EDM AMENDMENT #003

Effect of Ketamine in HD-Intoxicated Swine.

At one and three hours post-HD exposure, the animals were treated with bolus doses of ketamine (100 and 50 mg, iv, respectively). No effects from this treatment were observed in the Sa_{O2}, temperature or the heart rate of intoxicated animals. However a profound and prolonged apnea was induced in every animal with each ketamine challenge (Fig. 2, Table 1). Concomitant with the apnea induced by 100 mg ketamine challenges was a marked elevation of the blood pressure (30 min) which returned to baseline values (60 min). After 50 mg challenges, blood pressure was increased after one hour. The experiment was not continued for long enough time periods to assess whether blood pressure would decline to baseline values. No significant changes in ChE were noted and the urine output was 2.7 +/- 0.7 ml/kg/min. The animal was manually ventilated with 100 % oxygen if and when the pulse oximeter value decreased to 85 %. If manual ventilation was required more than twice the animal was ventilated with a Harvard ventilator.

Effect of HD-Exposure during Ketamine Anaesthesia

After one hour of steady-state ketamine anaesthesia (0.5 mg/kg/min) the animals were treated with HD (1 mg/kg). When compared to baseline values or ketamine exposure values only, no effects were observed with respect to Sa_{O2}, ET_{CO2}, respiration and blood pressure. No consistent changes in heart rate were obtained with respect to baseline values. However, HD exposure eliminated the decline in heart rate induced by ketamine anaesthesia. Muscle fasciculations were observed starting at 20-25 min following HD exposure and a more severe non-purposeful muscular activity was noted by 30-60 min post-HD treatment. These latter movements were judged to be overt convulsive activity, although we have no firm evidence that they were centrally driven. Treatment with diazepam (2 x 3 mg/kg, iv, 10 min interval) 2 h after HD treatment either failed to decrease convulsive activity, or transiently decreased it for less then 4 min. Concomitant with increased muscular activity the temperature of intoxicated animals generally increased. Cessation of ketamine anaesthesia and initiation of halothane anaesthesia eliminated all non-purposeful muscular activity within 20 min. ChE values did not change significantly from control values and urine output was 2.5 +/- 0.7 ml/kg/min.

Discussion

Initial studies investigated the effects of iv infusions of HD (1 mg/kg) in halothane anaesthetised animals. A dose of HD that was judged would approximate a median lethal dose (LD50) was chosen; iv LD_{50} values historically range from 0.2 mg/kg to > 8 mg/kg in a variety of species. Observations of HD-intoxicated swine led to the conclusion that this dose was not supralethal. No obvious signs of distress were apparent visually and other than a slight depression in blood pressure that occurred (~ 15 %), and then stabilised, and a gradual increase in ET_{CO2} (~ 160 %, 5 h) the animals did not appear to be severely poisoned. Although, from the literature, these signs were consistent with what one would expect from mild systemic HD intoxication, they would not be grounds for grave concern by an attending physician or anaesthesiologist. These same animals were then treated with the muscle relaxant, SCh at 5 h post-HD. Predictably, these animals became apneic. However, the apnea was extremely prolonged.

Clearly, SCh was contraindicated in these apparently mildly poisoned animals.

Additional studies examined the potential interactions of ketamine and HD. This drug is commonly used as an iv induction agent in the battlefield and there are reasons to believe, due to the fact that it is useful in relieving bronchoconstriction, supports the cardiovascular system during shock and produces only mild CNS depression, that it may be a useful anaesthetic to use with organophosphate nerve agent intoxicated patients. Pigs were treated with HD and 1 h later ketamine (iv, 100 mg) was introduced. This treatment resulted in an immediate apnea that lasted up to 23 min. At 3 h post-HD ketamine was repeated at a lower

THE INTERACTION OF CHEMICAL AGENTS AND THERAPEUTIC COMPOUNDS WITH DRUGS USED DURING ANAESHESIA
W7702-7-R627/001/EDM AMENDMENT #003

dose (iv, 50 mg) and similar results were once again obtained. In all cases, respiratory stability either did not return, or returned only very slowly

It thus appears that ketamine is potentially lethal in HD-poisoned pigs that appear normal other than exhibiting minimally depressed blood pressure

We further examined this interaction by reversing the order of drug/agent introduction into the pig Animals were placed under ketamine drip anaesthesia (0.5 mg/kg/min) and then exposed to HD (1 mg/kg) 1 h later. Within
minutes, muscle fasciculations were observed which rapidly progressed to what was judged to be outright convulsive activity. Diazepam injections (iv, 2 x 3 mg, 10 min interval) either failed to moderate this activity or only transiently (< 4 min) decreased it Cessation of ketamine and initiation of halothane anaesthesia dramatically decreased or eliminated all non-purposeful activity.

The interaction between SCh and HD was not altogether unexpected. Prolonged SCh-induced paralysis has been previously documented in-patients whose pseudocholinesterase has been inhibited by organophosphate insecticides or carbamate anticholinesterases HD has been shown to have anticholinesterase activity and at the low dose that was used inhibition was indeed obtained at the time of SCh administration. However, significant ChE activity (> 50 %) was still present at this time and it may be that as yet undefined interactions not involving the inhibition of this enzyme may be contributing towards the prolonged apnea occurring in these animals

The results obtained with ketamine and HD are more puzzling. Although it is conceivable that we are actually looking at an interaction between HD, ketamine *and* halothane, we believe this to be highly unlikely. In halothane-anaesthetised pigs, we observed the expected signs of systemic HD intoxication, but none of the signs exhibited in ketamine/HD treated animals Furthermore, the respiratory difficulties and muscular activity were very similar to those observed in experiments in which ketamine-only anaesthetised rats were treated with HD (unpublished data). The fact that the order of administration played an important role in determining the signs of intoxication, either prolonged apnea or respiratory distress or fasciculations progressing to what appeared to be frank convulsions is intriguing While both HD and ketamine have been shown to have anticholinesterase activity, results did not indicate that synergism was taking place between these drugs with respect to the inhibition of this enzyme. In fact, at the very low doses of ketamine used (22mg/kg is the commonly used iv induction dose for swine) no AChE depression was observed and in those experiments in which HD and ketamine were combined, AChE varied only slightly from baseline control values Although the experiments where ketamine anaesthetised animals were exposed to HD are obviously not relevant to a practical medical situation, they do provide additional information that points to an adverse interaction of ketamine and HD. Whether the observed induced muscular activity is driven peripherally or centrally is of yet not known with certainty.

Further work involving a more extensive analysis of clinical diagnostic parameters, as well as careful *in vitro* studies will doubtless be required to elucidate how these ketamine and HD interact with each other and whether this interaction takes place at a peripheral or central level.

In summary,

In HD-poisoned animals, ketamine and SCh can rapidly induce potentially life-threatening situations.

While the time to onset and action of SCh were normal in these studies, the duration of paralysis precludes its routine utilisation and could adversely impact on post-operative care and on available manpower and equipment resources.

THE INTERACTION OF CHEMICAL AGENTS AND THERAPEUTIC COMPOUNDS WITH DRUGS USED DURING ANAESHESIA
W7702-7-R627/001/EDM AMENDMENT #003

The use of ketamine in HD-intoxicated animals may produce an unexpected and prolonged apnea.

Furthermore, these same animals may also exhibit neuromuscular phenomena that would result in completely unsatisfactory operating conditions for surgery.

THE INTERACTION OF CHEMICAL AGENTS AND THERAPEUTIC COMPOUNDS WITH DRUGS USED DURING ANAESHESIA
W7702-7-R627/001/EDM AMENDMENT #003

Collection of Epidermal Dermal Samples for Histopathological Assessment post-Reactive Skin Decontaminant Lotion (RSDL):

(Animal Reference-97002, 003, 98034, 036, 037, 049, 053, 101-104, 99130)

Full-thickness (FT) specimens of porcine skin were obtained.

Method:

A disposable 8 mm punch was utilized to create 4 aseptic surgical wounds in the right lower quadrant (RLQ) of the abdomen of each of 5 animals RSDL was applied topically to 3 of these wounds at time zero Sterile normal saline (N/S) was applied to the 4th wound (control) at time zero A sterile non-occlusive military field-dressing covered the wounds.

At 2, 4 and 6 hour's post-application of RSDL the skin around the wounds was excised aseptically. Skin from around the control wound was excised aseptically at 6 hours post-application of N/S All specimens were placed in containers of 10% non-buffered formalin (10% NBF)

For further information on disposition of specimens/results of histopathological examination, contact Dr. MG Hamilton ((403) 544-4709)

THE INTERACTION OF CHEMICAL AGENTS AND THERAPEUTIC COMPOUNDS WITH DRUGS USED DURING ANAESHESIA
W7702-7-R627/001/EDM AMENDMENT #003

The Effect of OP Intoxication on the Action of Intravenous Induction agents:

(Animal Reference 99110-126)

Equipotent doses of ketamine (100 mg), thiopental (50 mg) and propofol (10 mg) representing approximately 1/3rd of the intravenous (iv) induction dose for swine were administered to swine anaesthetized with isoflurane Heart rate (HR), mean arterial pressure (MAP), temperature (°C), respiratory frequency (f), pulse oximetry (SpO_2) and capnography ($ETCO_2$) were measured. Following stabilization after exposure to GB (9 µg/kg over min), further doses of each drug were given.

PIG #	DRUG	WEIGHT (kg)	INTAKE (cc/kg/h)	OUT (cc/kg/h)	ONSET of APNEA	SPONT BREATH	REC.
110	Ketamine	21.9	7 24	1 27	51s	270s	1080s
111	Ketamine	20.3	7 52	1.50	85s	325s	2100s
112	Ketamine	20 8	7 93	2.90	55s	169s	1150s
113	Ketamine	21.5	8 40	2 99			
114	Ketamine	22 9	7.63	2.78			
115	Ketamine	19.1	7.59	2 11			
116	Ketamine	19.1	9.46	2 25			
117	Ketamine	17.3	7 36	2 84			
118	Ketamine	19.9	7 52	8 42			
119	Ketamine	18.1	7 70	7 28			
AVG Mean +/-SD	Ketamine	20.1 +/- 1 75 n=10	7.84 +/- 0.66 n=10	3.43 +/- 2.42 n=10	63 7 +/- 18 58 n=3	254 7 +/- 79 12 n=3	1443 3 +/- 569 77 n=3

Control values following ketamine 100 mg iv

PIG #	DRUG	WEIGHT (kg)	INTAKE (cc/kg/h)	OUT (cc/kg/h)	ONSET of APNEA	SPONT. BREATH	REC
120	STP	23 5	7.49	3 25	59s	240s	888s
121	STP	21.3	4.94	1.40	never	always	281s
122	STP	20 1	6 76	2 40	never	always	558s
123	STP	20.5	7.67	2.53	79s	195s	209s
AVG Mean +/-SD	STP	21 4 +/- 1 52 n=4	6 75 +/- 1.25 n=4	2.38 +/- 0 76 n=4	69.0 +/- n=2[1]	217 5 +/- n=2[1]	484 0 +/- 308.50 n=4

Control values following sodium thiopental (STP) 50 mg iv
[1] 2 of 4 animals *never* went apneic and *always* had spontaneous respirations

THE INTERACTION OF CHEMICAL AGENTS AND THERAPEUTIC COMPOUNDS WITH DRUGS USED DURING ANAESHESIA
W7702-7-R627/001/EDM AMENDMENT #003

PIG #	DRUG	WEIGHT (kg)	INTAKE (cc/kg/h)	OUT (cc/kg/h)	ONSET of APNEA	SPONT BREATH	REC.
124	propofol	21 1	7.61	2.98	Never[2]	Always[2]	149s
125	propofol	21.7	7.66	1.38	Never[2]	Always[2]	120s
126	propofol	19.7	7.28	2.17	Never[2]	Always[2]	Always[3]
AVG Mean +/-SD	Propofol	20.8 +/- 1 03 n=3	7.52 +/- 0 21 n=3	2.18 +/- 0 80 n=3	never +/- n=	always +/- n=	134.5 +/- n=2

Control values following propofol 10 mg iv
[2] 3 of 3 animals *never* went apneic and *always* had spontaneous respirations
[3] 1 of 3 animals never had an increase in ET_{co2} > 5 mm Hg

	DRUG	WEIGHT	IN	OUT	ONSET	SPONT	REC
AVG Mean +/-SD	Ketamine (100 mg)	20.1 +/- 1.75 n=10	7 84 +/- 0.66 n=10	3 43 +/- 2.42 n=10	63.7 +/- 18 58 n=3	254.7 +/- 79 12 n=3	1443.3 +/- 569 77 n=3
AVG Mean +/-SD	STP (50 mg)	21.4 +/- 1.52 n=4	6.75 +/- 1 25 n=4	2 38 +/- 0 76 n=4	69 0 +/- n=2[1]	217.5 +/- n=2[1]	484.0 +/- 308 50 n=4
AVG Mean +/-SD	Propofol (10 mg)	20 8 +/- 1.03 n=3	7 52 +/- 0.21 n=3	2.18 +/- 0 80 n=3	never +/- n=0[2]	always +/- n=0[2]	134.5 +/- n=2[3]

[1] 2 of 4 animals *never* went apneic and *always* had spontaneous respirations
[2] 3 of 3 animals *never* went apneic and *always* had spontaneous respirations
[3] 1 of 3 animals never had an increase in ET_{co2} > 5 mm Hg

Conclusions:
Propofol appears to be the least respiratory depressant of the available iv induction agents in control animals

THE INTERACTION OF CHEMICAL AGENTS AND THERAPEUTIC COMPOUNDS WITH DRUGS USED DURING ANAESHESIA
W7702-7-R627/001/EDM AMENDMENT #003

Following an arbitrary period of approximately 70 minutes (4200s) post-GB, and no matter what the stage of recovery, the same dose of iv induction agent was administered. 2nd and 3rd doses were administered following recovery or return of respiratory stability.

Pig #	Drug	Recov.	Onset	Spont.	Rec	Onset	Spont	Rec	Onset	Spont.	Rec
110	Ket.	4020s	75s	325s	3300s	90s	780s	never	58	never	never
111	Ket	4320s	80s	343s	4260s	109s	366s	4200s	-	-	-
112	Ket	4260s	78s	160s	1576s	never	always	300	-	-	-
113	Ket.										
114	Ket.										
115	Ket										
116	Ket.										
117	Ket										
118	Ket.										
119	Ket										
AVG Mean +/-SD	KET	4200s +/- 158 7 n=3	77 7 +/- 2 52 n=3	276.0 +/- 100.86 n=3	3045.3 +/- 1360.0 n=3						

Values following ketamine 100 mg iv in GB-intoxicated swine
Note. only first dose post-GB/control swine

Pig #	Drug	Recov	Onset	Spont	Rec	Onset	Spont	Rec	Onset	Spont	Rec
120	STP	4200s	30s	256s	Never	?	101s	?+	?	never	never
121	STP	4260s	never	always	174	never	always	110s	58s	125s	181s
122	STP	4200s	82	1553	2760	70s	153s	never	65s	never	never
123	STP	4200s	50	207	3600+	45s	150s	4680+	145s	147s	205s
AVG Mean +/-SD	STP										

\+ required manual ventilation

THE INTERACTION OF CHEMICAL AGENTS AND THERAPEUTIC COMPOUNDS WITH DRUGS USED DURING ANAESHESIA
W7702-7-R627/001/EDM AMENDMENT #003

Pig #	Drug	Recov.	Onset	Spont.	Rec.	Onset	Spont	Rec.	Onset	Spont	Rec.
124	propof	4140s	never	always	never+	never	always	always	never	always	581s
125	propof	4200s	never	always	794s	never	always	678s	never	always	412s
126	propof	4440s	never	always	?	never	always	367s	never	always	149s
AVG Mean +/-SD	Propof										

\+ required manual ventilation

PIG #	DRUG	ONSET	SPONT.	REC.	*Onset*	*Spont*	*Rec*
110	Ketamine	51s	270s	1080s	*75s*	*325s*	*3300s*
111	Ketamine	85s	325s	2100s	*80s*	*343s*	*4260s*
112	Ketamine	55s	169s	1150s	*78s*	*160s*	*1576s*
113	Ketamine						
114	Ketamine						
115	Ketamine						
116	Ketamine						
117	Ketamine						
118	Ketamine						
119	Ketamine						
AVG Mean +/-SD	Ketamine	63.7 +/- 18 58 n=3	254 7 +/- 79 12 n=3	1443 3 +/- 569.77 n=3	*77.7 + -2 52 n=3*	*276.0 + -100.86 n=3*	*3045.3 - - 1360 0n=3*

Comparison of effects of ketamine before and after GB 9 µg/kg
Note post-GB in *italics*

THE INTERACTION OF CHEMICAL AGENTS AND THERAPEUTIC COMPOUNDS WITH DRUGS USED DURING ANAESHESIA
W7702-7-R627/001/EDM AMENDMENT #003

PIG #	DRUG	ONSET	SPONT	REC.	*Onset*	*Spont.*	*Rec.*	
120	STP	59s	240s	888s	*30s*	*256s*	*Never-*	
121	STP	never	always	281s	*never*	*always*	*174*	
122	STP	never	always	558s	*82s*	*1553s*	*2760s*	
123	STP	79s	195s	209s	*50s*	*207s*	*3600s-*	
AVG Mean +/-SD	STP	69 0 +/- n=2[1]	217.5 +/- n=2[1]	484.0 +/- 308.50 n=4	*54 0* *+ -26 22* *n=3[2]*	*672 0* *+ -763.36* *n=3[2]*	- - *n=2[3]*	

Comparison of effects of STP before and after GB 9 µg/kg
Note: post-GB in *Italics*

[1] 2 of 4 animals *never* went apneic and *always* had spontaneous respirations
[2] 1 of 4 animals *never* went apneic and *always* had spontaneous respirations
+ required manual ventilation/never recovered

PIG #	DRUG	ONSET	SPONT	REC	Onset	Spont.	Rec
124	propofol	never[1]	always[1]	149s	*Onset*	*Spont*	*Rec*
125	propofol	never[1]	always[1]	120s	*never*	*always*	*never-*
126	propofol	never[1]	always[1]	always[2]	*never*	*always*	*794s*
					never	*always*	*?*
AVG Mean +/-SD	Propofol	never +/- n=	always +/- n=	134.5 +/- n=2	*never* + - *n=0[1]*	*always* + - *n=0[1]*	*134.5* - - *n=2[3]*

Comparison of effects of propofol before and after GB 9 µg/kg
Note: post-GB in *italics*

[1] 3 of 3 animals *never* went apneic and *always* had spontaneous respirations
[2] 1 of 3 animals never had an increase in ET_{co2} > 5 mm Hg
[23] 1 of 3 animals never recovered (See following 2nd and 3rd doses)

Conclusion:
All the iv induction agents exert a greater respiratory depressant effect post-GB with propofol being the least depressant.

THE INTERACTION OF CHEMICAL AGENTS AND THERAPEUTIC COMPOUNDS WITH DRUGS USED DURING ANAESHESIA

W7702-7-R627/001/EDM AMENDMENT #003

Collection of Blood for Evaluation of Possible Dyscrasias post-OP post-Therapeutic Agents

(Animal Reference-98066-071)

Preliminary evaluation of blood dyscrasias as an etiological factor in Gulf War Syndrome (GWS) was undertaken as a collaborative effort between DRES and Dr. Martha Lyons (U. Sask.)

The timed effect of pyridostigmine, tacrine and metrifonate on coagulation profiles was assessed in the DSM.

At the time of this report results are pending. Further information may be obtained from Dr. MG Hamilton

THE INTERACTION OF CHEMICAL AGENTS AND THERAPEUTIC COMPOUNDS WITH DRUGS USED DURING ANAESHESIA
W7702-7-R627/001/EDM AMENDMENT #003

Collection of Thoracic Contents For Analysis of Potential Free Radical Condensates

(Animal Reference-98053-074)

Cardiomyopathy associated with OPs may be either primary or secondary DR. S Baskin, who is seeking an association between convulsive activity and cardiomyopathy, suspects the latter.

To assist Dr. Baskin the thoracic contents (pluck) were harvested *post-mortem*, frozen at -80°C and shipped on "dry ice" to Dr. Baskin. Blood was collected *ante-mortem* in the control period and immediately prior to termination.

DATE	ANIMAL #	OP	OTHER	CONVULSION
98/06/15	98-053	GB-9 µg/kg		yes
98/06/16	98-054	"		yes
98/06/17	98-055	"		?
98/06/18	98-056			no
98/06/22	98-057	GB-9 µg/kg		yes
98/06/23	98-058	"		?
98/06/24	98-059	"		yes
98/06/25	98-060	"		?
98/06/29	98-061	"		yes
98/06/30	98-062	"		?
98/07/07	98-063			no
98/07/08	98-064	GB- 9 µg/kg		?
98/07/09	98-065			no
98/07/14	98-066			no
98/07/14	98-067			no
98/07/15	98-068		tacrine	no
98/07/15	98-069			no
98/07/16	98-070		tacrine	no
98/07/16	98-071		metronifate	no
98/07/20	98-072	xxxx		yes
98/07/21	98-073	xxxx		?
98/07/22	98-074	xxxx		?

THE INTERACTION OF CHEMICAL AGENTS AND THERAPEUTIC COMPOUNDS WITH DRUGS USED DURING ANAESHESIA
W7702-7-R627/001/EDM AMENDMENT #003

Determination of IV Dose to Maintain Therapeutic Levels post-Autoinjector (AI)·

(Animal Reference 99137-145, 147-151, 162-179)

OP poisoning may require both immediate and prolonged therapy. Immediate therapy may be administered at the scene of injury with hope of improving chances of survival until the victim can be given further definitive medical care.

The first medical therapy in cases of serious nerve agent poisoning is provided by an autoinjector containing a mixture of 2 active components. Atropine protects against the excess of ACh formed and HI-6 is the acetylcholinesterase (AChE) oxime reactivator. HI-6 has proven effective in the treatment of soman (GD), an "oxime-resistant" OP

The doses of atropine sulphate (2 mg) and HI-6 (500 mg) administered im in this study were approximately equivalent to the maximum dose deliverable to a 70 kg human.

Therapeutic Levels of HI-6 post-AI

(Animal Reference 99158-161)

Each animal received the equivalent of 1 AI im in the right thigh Blood samples for HI-6/ChE determination were obtained at 30s, 1,2,3, 5, 10, 15, 20, 25, 30min post-AI and every 15 min thereafter until 5h had elapsed post AI

Serum samples were analyzed for HI-6 using an HPLC (Hewlett-Packard, 1100 Series).

For further information on HPLC results, contact Dr. MG Hamilton ((403) 544-4709).

Determination of Intravenous Infusion Rate to Maintain Therapeutic Levels of HI-6 post-AI·

(Animal Reference 99162-173)

12 animals were divided into 4 groups of 3 animals each·

01)-Each animal received the equivalent of 1 AI im in the right thigh 15m later HI-6 was infused at a rate of 10 mg/hr for 5h. Blood samples for HI-6/ChE determination were obtained at 30s, 1,2,3, 5, 10, 15, 20, 25, 30min post-AI and every 15 min thereafter until 5h had elapsed post AI.
02)- Each animal received the equivalent of 1 AI im in the right thigh 15m later HI-6 was infused at a rate of 40 mg/hr for 5h. Blood samples for HI-6/ChE determination were obtained at 30s, 1,2,3, 5, 10, 15, 20, 25, 30min post-AI and every 15 min thereafter until 5h had elapsed post AI.
03)- Each animal received the equivalent of 1 AI im in the right thigh 15m later HI-6 was infused at a rate of 60 mg/hr for 5h Blood samples for HI-6/ChE determination were obtained at 30s, 1,2,3, 5, 10, 15, 20, 25, 30min post-AI and every 15 min thereafter until 5h had elapsed post AI.
04)- Each animal received the equivalent of 1 AI im in the right thigh. 15m later HI-6 was infused at a rate of 80 mg/hr for 5h. Blood samples for HI-6/ChE determination were obtained at 30s, 1,2,3, 5, 10, 15, 20, 25, 30min post-AI and every 15 min thereafter until 5h had elapsed post AI

Serum samples were analyzed for HI-6 using an HPLC (Hewlett-Packard, 1100 Series)

For further information on HPLC results, contact Dr MG Hamilton ((403) 544-4709)

THE INTERACTION OF CHEMICAL AGENTS AND THERAPEUTIC COMPOUNDS WITH DRUGS USED DURING ANAESHESIA
W7702-7-R627/001/EDM AMENDMENT #003

Efficacy of AI/Intravenous HI-6 in the Prevention of Sign(s) caused by Soman (GD) Intoxication:

(Animal Reference 99174-179)

GD was administered iv starting at 9 µg/kg At the onset of "signs", an AI (equivalent) was administered im. Fifteen (15) min. later, an iv infusion of HI-6 at 60 mg/hr was started and continued for 5 hours. Because of adverse respiratory effects (apnea), GD was administered in lower doses of 5 µg/kg and 2.5 µg/kg to ascertain the final test dose of GD.

Subsequently, GD was administered at 2.5 µg/kg over 5 min With the onset of signs, 1 AI equivalent im was administered followed in 15 min by HI-6 at a rate of 60 mg/hr for 5h.

Blood samples for HI-6/ChE determination were obtained at 30s, 1,2,3, 5, 10, 15, 20, 25, 30min post-AI and every 15mn thereafter until 5h had elapsed post AI

Serum samples were analyzed for HI-6 using an HPLC (Hewlett-Packard, 1100 Series)

For further information on HPLC results, contact Dr. MG Hamilton ((403) 544-4709).

Discussion:

During the course of our investigations we have noted significant disparities between our findings (unpublished) and work published by Nyberg et al

In brief, they found that one autoinjector administered IM to anaesthetized domestic swine counteracted the signs of OP poisoning (soman-2xLD_{50}), obviated the need for ventilatory assistance and reactivated acetylcholinesterase (AChE)

In contrast, we have observed that one autoinjector (equivalent) administered IM to anaesthetized domestic swine did not counteract the signs of OP poisoning with GD, did not obviate the need for ventilatory assistance and did not reactivate ChE

There are both *similarities* and *differences* in the methodology:

Similarities in methodology.
01-castrated male domestic swine
02-weight approx 22 kg
03-similar instrumentation

Differences in methodology.

01-premedication	-azaperone IM (S)	vs. unpremedicated (C)
02-anaesthetic agent	-ketamine (S)	vs. isoflurane (C)
03-GD dosage	-9 :g/kg IV (S)	vs 2.5 :g/kg IV (C)
04-GD infusion time	-over 20 mn (S)	vs. over 5 mn (C)
05-antidote	-binary autoinjector (S)	vs. intramuscular (C)
06-antidote timing	-5 mn. into GD infusion (S)	vs. with signs (C)

In the Swedish study animals received approximately 50 µg of GD prior to administration of the antidote, whereas in our study animals received slightly more than 50 µg of GD prior to administration of the antidote. Animals in the Swedish study demonstrated signs of OP intoxication prior to the administration of the antidote.

THE INTERACTION OF CHEMICAL AGENTS AND THERAPEUTIC COMPOUNDS WITH DRUGS USED DURING ANAESHESIA
W7702-7-R627/001/EDM AMENDMENT #003

Conclusion:
Pigs in the Swedish and Canadian studies received equivalent amounts of GD prior to the administration of antidote.

Animals in the Swedish study received the antidote via a binary autoinjector supplied by the manufacturer (Astra Tech AB, Sweden). Canadian animals received the identical drugs via an IM injection There are clear advantages to the autoinjector but studies in Sweden have demonstrated that a satisfactory level of HI-6 (4 $\mu g\ mL^{-1}$) is attained within 1 min. following either conventional IM or autoinjector administration

We have used autoinjectors (old) and IM administration of antidote in GB intoxicated animals with comparable rates of reactivation of ChE.

Conclusions:
The influence of route of administration on the observed differences in both morbidity and enzyme reactivation should be minimal.

The swine in the Swedish study were premedicated with azaperone (Stresnil, Janssen) and received iv ketamine whereas the animals in the Canadian study had an inhalation induction with halothane and were subsequently maintained on isoflurane.

Ketamine, *in vitro*, reversibly inhibits ChE and theoretically could prevent the action of an irreversible inhibitor like GD on ChE Our data suggests a decrease in CHe activity with isoflurane general anaesthesia (compared to halothane controls).

The influence of ketamine as a NMDA channel blocker in these circumstances remains to be elucidated

The influence of azaperone premedication (if any) is unknown

Conclusion:
The efficacy of the antidote with respect to both clinical course and ChE reactivation appears to be influenced by the anaesthetic agent.

THE INTERACTION OF CHEMICAL AGENTS AND THERAPEUTIC COMPOUNDS WITH DRUGS USED DURING ANAESHESIA
W7702-7-R627/001/EDM AMENDMENT #003

RECOMMENDATIONS:

Inappropriate anaesthetic agent selection for the care of patients who have been poisoned by OP agents may increase morbidity and mortality The effectiveness of the antidote issued to the Canadian Forces appears suspect under some conditions which might occur.

It is suggested that further investigation be conducted to determine:

01-the influence of butyrophenone premedication on clinical course and enzyme reactivation
Azaperone is not used in human anaesthesia. Droperidol should be considered.
02-the influence of IV ketamine on clinical course and enzyme reactivation

Suggested Animal Utilization.

01-compare the influence of IM azaperone premedication (3.3 mg/kg) vs no premedication in animals induced with halothane and converted to IV ketamine (0.5 mg/kg/min).
a) if azaperone has a significant effect, the experiment should be repeated using isoflurane
b) if azaperone has a significant effect with isoflurane, the experiment should be repeated with droperidol.

Premed (no/yes) → halothane x 60 min (ChE q5min) → maintenance anaesthetic x 90 min (ChE q5mm) → GD (2 5 :g/kg/5 min)

→ Antidote (signs) → (ChE q30 sec x 5min, q5min until 30 min q 15 min until 5 hr post-GD)

EXPERIMENT #	CONDITIONAL UPON RESULTS	PREMED	MAINTENANCE	ANIMAL #
1	-	none	IV ketamine	3
2	-	azaperone	IV ketamine	3
3	1,2	azaperone	isoflurane	3
4	3	droperidol	isoflurane	3

Physiological parameters would be measured, collated and analyzed

THE INTERACTION OF CHEMICAL AGENTS AND THERAPEUTIC COMPOUNDS WITH DRUGS USED DURING ANAESHESIA
W7702-7-R627/001/EDM AMENDMENT #003

Acknowledgments·

The contractor acknowledges with gratitude the assistance of the DRES vivarium staff (L.Schofield, DVM, G.Rayner, G.Schnell, D.Risk)